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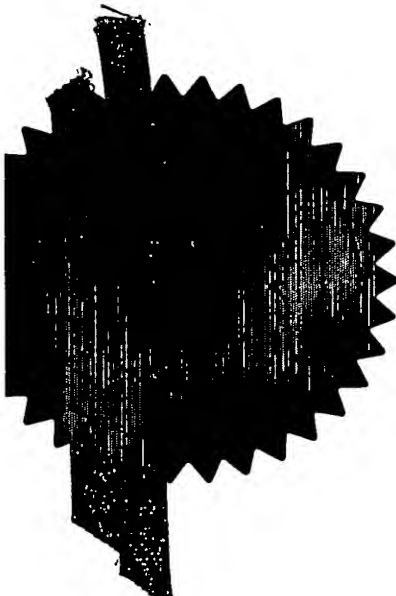
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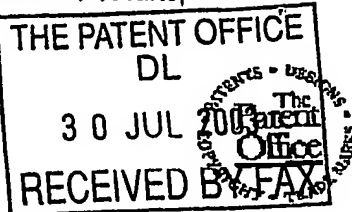
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Request for grant of a patent

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1. Your reference PN0360 GB

0317815.9

2. Patent application number
(The Patent Office will fill in this part)

30 JUL 2003

3. Full name, address and postcode of the or of each applicant (underline all surnames)

AMERSHAM HEALTH AS
Nycoven 2
Postboks 4220 Nydalen
N-0401 Oslo
Norway

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

Norway

8673864001

4. Title of the invention

IMAGING AGENTS

5. Name of your agent (if you have one)

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

HAMMETT, Audrey, Grace, Campbell ; ROLLINS, Anthony, John;
HAMMER, Catriona, MacLeod and BRYAN, Ian, Bennett
Amersham plc
Amersham Place
Little Chalfont
Buckinghamshire HP7 9NA

Patents ADP number (if you know it)

8189375004

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country

Priority application number
(if you know it)Date of filing
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7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
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8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

Yes

- a) any applicant named in part 3 is not an inventor, or
 - b) there is an inventor who is not named as an applicant, or
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Continuation sheets of this form

Description 18

Claim(s) 5 DL

Abstract 0

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Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 8/77) 1 /

Request for substantive examination (Patents Form 10/77)

Any other documents (please specify)

11.

HAMMETT, Audrey, Grace, Cambell

I/We request the grant of a patent on the basis of this application.

Signature

Date

30 July 2003

12. Name and daytime telephone number of person to contact in the United Kingdom HALLS, Jennie 01494 542032

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DUPLICATE

IMAGING AGENTS

The present invention relates to new peptide-based compounds and their use for
5 diagnostic imaging techniques such as positron emission tomography (PET).
More specifically the invention relates to the use of such peptide-based
compounds as targeting vectors that bind to receptors associated with
angiogenesis, in particular integrin receptors, for example, the $\alpha v \beta 3$ integrin
receptor. Such compounds may thus be used for diagnosis of, for example,
10 malignant diseases, heart diseases, endometriosis, inflammation-related diseases,
rheumatoid arthritis and Kaposi's sarcoma.

New blood vessels can be formed by two different mechanisms: vasculogenesis or
angiogenesis. Angiogenesis is the formation of new blood vessels by branching
15 from existing vessels. The primary stimulus for this process may be inadequate
supply of nutrients and oxygen (hypoxia) to cells in a tissue. The cells may
respond by secreting angiogenic factors, of which there are many; one example,
which is frequently referred to, is vascular endothelial growth factor (VEGF). These
factors initiate the secretion of proteolytic enzymes that break down the proteins of
20 the basement membrane, as well as inhibitors that limit the action of these
potentially harmful enzymes. The other prominent effect of angiogenic factors is
to cause endothelial cells to migrate and divide. Endothelial cells that are
attached to the basement membrane, which forms a continuous sheet around
blood vessels on the contraluminal side, do not undergo mitosis. The combined
25 effect of loss of attachment and signals from the receptors for angiogenic factors
is to cause the endothelial cells to move, multiply, and rearrange themselves, and
finally to synthesise a basement membrane around the new vessels.

Angiogenesis is prominent in the growth and remodelling of tissues, including
30 wound healing and inflammatory processes. Tumours must initiate angiogenesis
when they reach millimetre size in order to keep up their rate of growth.
Angiogenesis is accompanied by characteristic changes in endothelial cells and

their environment. The surface of these cells is remodelled in preparation for migration, and cryptic structures are exposed where the basement membrane is degraded, in addition to the variety of proteins which are involved in effecting and controlling proteolysis. In the case of tumours, the resulting network of blood vessels is usually disorganised, with the formation of sharp kinks and also arteriovenous shunts. Inhibition of angiogenesis is also considered to be a promising strategy for antitumour therapy. The transformations accompanying angiogenesis are also very promising for diagnosis, one example being malignant disease, but the concept also shows great promise in inflammation and a variety of inflammation-related diseases, including atherosclerosis, the macrophages of early atherosclerotic lesions being potential sources of angiogenic factors.

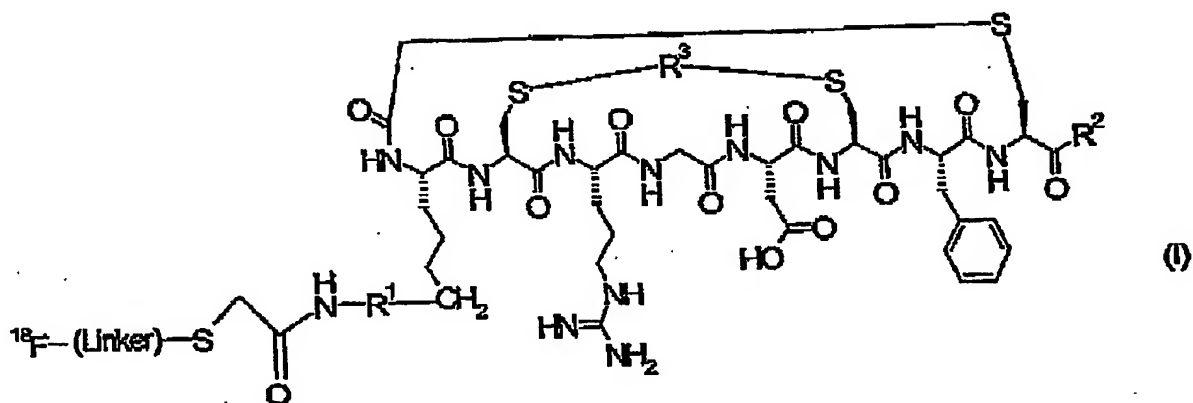
Many ligands involved in cell adhesion contain the tripeptide sequence arginine-glycine-aspartic acid (RGD). The RGD sequence appears to act as a primary recognition site between the ligands presenting this sequence and receptors on the surface of cells. It is generally believed that secondary interactions between the ligand and receptor enhance the specificity of the interaction. These secondary interactions might take place between moieties of the ligand and receptor that are immediately adjacent to the RGD sequence or at sites that are distant from the RGD sequence.

The efficient targeting and imaging of integrin receptors associated with angiogenesis *in vivo* demands therefore a selective, high affinity RGD based vector that is chemically robust and stable. Furthermore, the route of excretion is an important factor when designing imaging agents in order to reduce problems with background.

WO 03/006491 describes peptide-based compounds which target integrin receptors associated with angiogenesis. However, there exists a need for further such peptide-based compounds having utility for diagnostic imaging techniques such as PET.

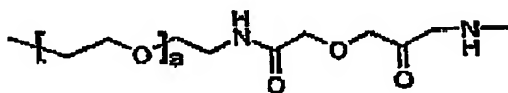
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Therefore, according to a first aspect of the invention, there is provided a compound of formula (I):



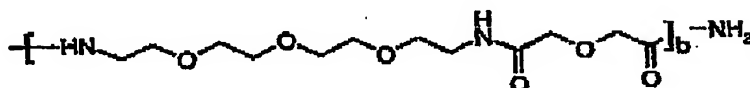
wherein

R^1 is either a bond or is



wherein a is an integer of from 1 to 30;

R^2 is



wherein b is an integer of from 0 to 10;

R^3 is a C_{1-4} alkylene or C_{2-4} alkenylene bridge;

the Linker is a C_{1-30} hydrocarbyl group optionally including 1 to 10 heteroatoms.

In the compounds of formula (I):

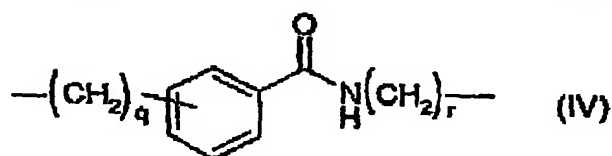
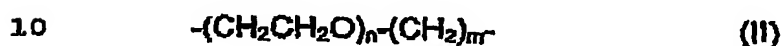
R^3 is preferably C_{1-4} alkylene, and more preferably $-\text{CH}_2-$;

a is preferably an integer of from 1 to 10, and is most preferably 5;

PN0360

b is preferably 1.

In formula (I) the Linker is a C_{1-30} hydrocarbyl group optionally including 1 to 10 heteroatoms such as oxygen or nitrogen, and may be chosen to provide good *in vivo* pharmacokinetics, such as favourable excretion characteristics. Suitable Linker groups include alkyl, alkenyl, alkynyl chains, aromatic, polyaromatic, and heteroaromatic rings, and polymers comprising ethyleneglycol, amino acid, or carbohydrate subunits. The Linker is preferably selected from (II), (III) and (IV):



wherein:

- 15 n is an integer of 1 to 20;
m is an integer of 1 to 10;
p is an integer of 1 to 20;
q is an integer of 0 to 4;
r is an integer of 1 to 10.

20

In formula (II), n is typically 2 to 6, suitably 3, and m is typically 1 to 4, suitably 2.

In formula (III), p is typically 1 to 6, suitably 3.

25 In formula (IV), the group $-(CH_2)_q-$ is suitably attached in the *para* position relative to the amide group, q is typically 0 to 4, suitably 1, and r is typically 1 to 4, suitably 2.

30 As shown in the *in vitro* competition binding assay below, the compounds of formula (I) bind to receptors associated with angiogenesis. These compounds

may thus be useful for *in vivo* diagnosis and imaging of diseases and conditions associated with angiogenesis.

5 The term "diseases and conditions associated with angiogenesis" includes those diseases and conditions referred to below. Reference is also made in this regard to WO 98/47541.

10 Diseases and conditions associated with angiogenesis include different forms of cancer and metastasis, for example, breast, skin, colorectal, pancreatic, prostate, lung or ovarian cancer.

Other diseases and conditions associated with angiogenesis are inflammation (for example, chronic inflammation), atherosclerosis, rheumatoid arthritis and gingivitis.

15 Further diseases and conditions associated with angiogenesis are arteriovenous malformations, astrocytomas, choriocarcinomas, glioblastomas, gliomas, hemangiomas (childhood, capillary), hepatomas, hyperplastic endometrium, ischemic myocardium, endometriosis, Kaposi sarcoma, macular degeneration, melanoma, neuroblastomas, occluding peripheral artery disease, osteoarthritis,
20 psoriasis, retinopathy (diabetic, proliferative), scleroderma, seminomas and ulcerative colitis.

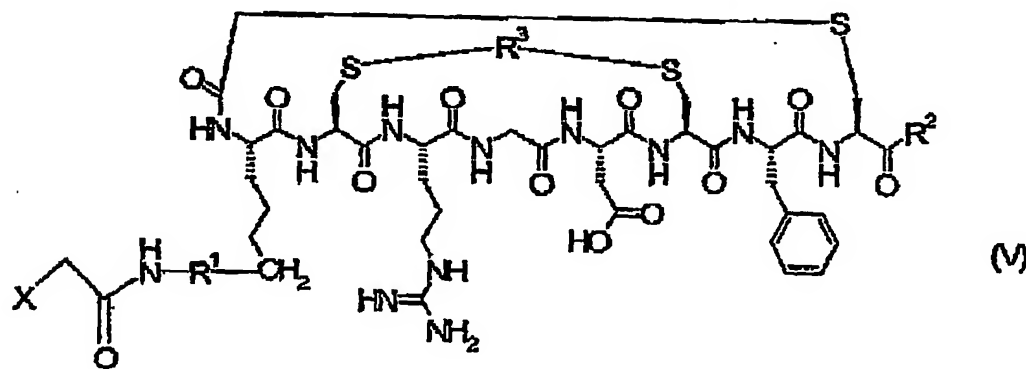
Therefore, according to a further aspect of the invention, there is provided a compound of formula (I) for use in medicine, particularly in the *in vivo* diagnosis or
25 imaging, for example by PET, of a disease or condition associated with angiogenesis.

30 In the alternative, there is provided a method for *in vivo* diagnosis or imaging of a disease or condition associated with angiogenesis which comprises the step of administering a compound of formula (I) to a human or animal body, followed by generation of an image, suitably a PET image, of part or all of said body.

The compounds of formula (I) are preferably administered in a radiopharmaceutical formulation. A "radiopharmaceutical formulation" is defined in the present invention as a formulation comprising a compound of formula (I) in a form suitable for administration to a mammal, such as a human. Administration is preferably carried out by injection of the radiopharmaceutical formulation as an aqueous solution. Such a radiopharmaceutical formulation may optionally contain further ingredients such as buffers, pharmaceutically acceptable solubilisers (for example cyclodextrins or surfactants such as Pluronic, Tween, or phospholipids), pharmaceutically acceptable stabilisers or antioxidants (such as ascorbic acid, gentisic acid or para-aminobenzoic acid) or bulking agents for lyophilisation (such as sodium chloride or mannitol). The radiopharmaceutical formulation is administered in an amount which gives a reliable image, taking into account the nature of the disease or condition being investigated, size of patient, and such other factors as would be apparent to a person skilled in the art. For PET, a suitable amount of the compound of formula (I) is 0.1 to 100 mCi, preferably 1 to 20mCi.

Therefore, in a further aspect of the invention, there is provided a radiopharmaceutical formulation comprising a compound of formula (I) and one or more pharmaceutically acceptable excipients.

A compound of formula (I) may be prepared from the corresponding compound of formula (V):



wherein R^1 , R^2 , and R^3 are as defined for the compound of formula (I) and X is a leaving group selected from chloro, bromo, and iodo, and is preferably chloro; by reaction with the appropriate compound of formula (VI):

5



wherein the Linker is as defined for the compound of formula (I).

Compounds of formula (V) are novel and thus represent a further aspect of the present invention.

10

The reaction of compounds of formulae (V) and (VI) may be performed using the methodologies described in co-pending application PCT/GB03/01332. In general terms, the reaction may be effected in a suitable solvent, for example in an aqueous buffer in the pH range 5 to 11, and at a non-extreme temperature of from 5 to 70°C, preferably at ambient temperature.

15

Compounds of formula (V) may be prepared by standard methods of peptide synthesis, for example, solid-phase peptide synthesis, for example, as described in Atherton, E. and Sheppard, R.C.; "Solid Phase Synthesis"; IRL Press: Oxford, 1989. WO 03/006491 also describes synthesis of analogous peptides and in this regard, is incorporated herein by reference. Incorporation of the bridging group R^3 may be effected by reaction of the corresponding peptide containing two free thiol groups, with the relevant dichloroalkane or dichloroalkene (such as dichloromethane when R^3 is to be methylene). Incorporation of the group "X-CH₂C(O)-" in a compound of formula (V) may be achieved by reaction of the N-terminus or an amine-containing amino acid, preferably lysine, of the peptide with the reagent of formula (VII):

25

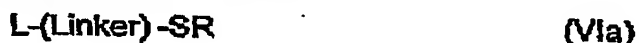
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under standard conditions for peptide bond formation; wherein X is as defined for

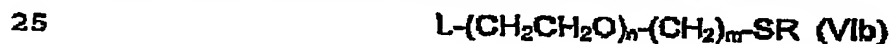
the compound of formula (V), and Z is -OH or a suitable activating group such as, chloro, bromo, fluoro, -OC(O)CH₂-X wherein X is as defined for the compound of formula (V), or when Z is -OH the acid may be activated using *in situ* agents such as 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) or N-[(dimethylamino)-1H-1,2,3-triazolo[4.5-b]pyridin-1-ylmethylene]-N-methylmethanaminium hexafluorophosphate N-oxide (HATU).

Compounds of formula (VI) may be prepared by standard methods such as those described in co-pending application PCT/GB03/01332, for example, from the corresponding compound of formula (Via):



wherein L is a leaving group such as p-toluenesulphonate, trifluoromethanesulphonate, or methanesulphonate, and the Linker is as defined for the compound of formula (VI) and R is hydrogen or a thiol protecting group; by reaction with cyclotron produced aqueous [¹⁸F]-fluoride, suitably pre-activated by evaporation from a base (for example, from tetrabutylammonium or K₂CO₃/Kryptofix-222), in a suitable solvent such as acetonitrile, N,N-dimethylformamide, or dimethyl sulphoxide, typically at elevated temperature, for example 60 to 120°C, followed by removal of any thiol protecting group using standard methods.

Compounds of formula (VI) in which the Linker is of formula (II) may be prepared from the corresponding compound of formula (Vib):



wherein L is a leaving group such as p-toluenesulphonate, trifluoromethanesulphonate, or methanesulphonate, and n and m are as defined for the formula (II) and R is hydrogen or a thiol protecting group; by reaction with cyclotron produced aqueous [¹⁸F]-fluoride, suitably pre-activated by evaporation from a base (for example, from tetrabutylammonium or K₂CO₃/Kryptofix-222), in a suitable solvent such as acetonitrile, N,N-

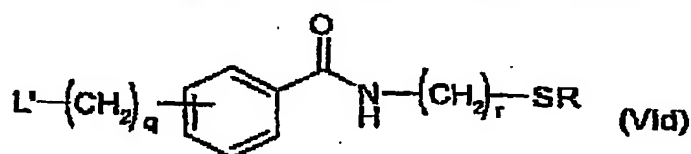
dimethylformamide, or dimethyl sulphoxide, typically at elevated temperature, for example 60 to 120°C, followed by removal of any thiol protecting group using standard methods.

- 5 Compounds of formula (VI) in which the Linker is of formula (III) may be prepared from the corresponding compound of formula (VIc):



- wherein L is a leaving group such as p-toluenesulphonate, trifluoromethanesulphonate, or methanesulphonate, and p is as defined for the formula (III) and R is hydrogen or a thiol protecting group;
- 10 by reaction with cyclotron produced aqueous [¹⁸F]-fluoride, suitably pre-activated by evaporation from a base (for example, from tetrabutylammonium or K₂CO₃/Kryptofix-222), in a suitable solvent such as acetonitrile, N,N-dimethylformamide, or dimethyl sulphoxide, typically at elevated temperature, for example 60 to 120°C, followed by removal of any thiol protecting group using standard methods.
- 15

- Compounds of formula (VI) in which the Linker is of formula (IV) may be prepared from the corresponding compound of formula (VId):
- 20



- wherein L' is a leaving group such as iodo, p-toluenesulphonate, trifluoromethanesulphonate, or methanesulphonate and when q is 0, L' can be nitro or an iodonium or ammonium salt, and q and r are as defined for the formula (IV) and R is hydrogen or a thiol protecting group;
- 25 by reaction with cyclotron produced aqueous [¹⁸F]-fluoride, suitably pre-activated by evaporation from a base (for example, from tetrabutylammonium or K₂CO₃/Kryptofix-222), in a suitable solvent such as acetonitrile, N,N-dimethylformamide, or dimethyl sulphoxide, typically at elevated temperature, for example 60 to 120°C, followed by removal of any thiol protecting group using
- 30

standard methods.

5 In formulae (Via), (Vib), (Vlc), and (Vld), suitable thiol protecting groups include (Phenyl)₃C- (trityl) and others as may be found described in Protecting Groups in Organic Synthesis, Theodora W. Greene and Peter G. M. Wuts, published by John Wiley & Sons Inc. Removal of such thiol protecting groups may be effected by standard methods, such as those described in Greene. For example, where R is trityl, the free thiol may be formed by treatment with dilute acid, for example trifluoroacetic acid in a chlorinated solvent, such as dichloromethane.

10

In one preferred aspect, the compounds of formulae (Via), (Vib), (Vlc), and (Vld) may be bound to a solid support, such as polymer beads or coatings, for example, a trityl or chlorotrityl resin. In this aspect, the excess reagents and by-products of the radio-fluorination reaction may be separated from the polymer-bound product by washing. Using the deprotection methods as described above, effects cleavage of the compound of formula (VI) from the solid support. This approach may be particularly suitable for automated production of the compounds of formula (VI). Alternatively, the by-products of thiol deprotection, where insoluble in the reaction mixture, may be removed by filtration.

15
20

According to a further aspect of the invention there is provided a kit for the preparation of a radiofluorinated peptide of formula (I) comprising a prosthetic group of formula (Via), (Vib), (Vlc), or (Vld) and an activated peptide of formula (V).

25

In use of the kit, the compound of formula (Via), (Vib), (Vlc), or (Vld) would be converted to the corresponding compound of formula (VI) using methods described above. Preferably, the compound of formula (VI) or a thiol protected precursor of any thereof, may be separated from waste reactants by passing the reaction mixture through a Solid Phase Extraction (SPE) cartridge. The SPE cartridge may comprise a graphite pad or C₁₈ stationary phase. Any thiol protecting group may be removed, for example, by addition of an acid such as

30

trifluoroacetic acid. Where the thiol group in the compound of formula (VI) is protected with a hydrophobic group, such as a trityl group, the deprotection may conveniently be effected on the SPE cartridge, whereby the hydrophobic thiol protecting group (such as trityl) remains bound on the stationary phase while the labelled prosthetic group of formula (VI) is eluted in high purity and yield. The compound of formula (VI) would then be added to the compound of formula (V) or which may suitably be dissolved in aqueous buffer (pH 7-11). After reaction at a non-extreme temperature for 1 to 60 minutes, the labelled peptide may be purified, for example, by SPE and collected.

The invention is illustrated by way of the following Examples, in which these abbreviations are used throughout:

DCM: dichloromethane

TFA: trifluoroacetic acid

THF: tetrahydrofuran

HBTU: 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

Boc: tert-butoxy-carbonyl

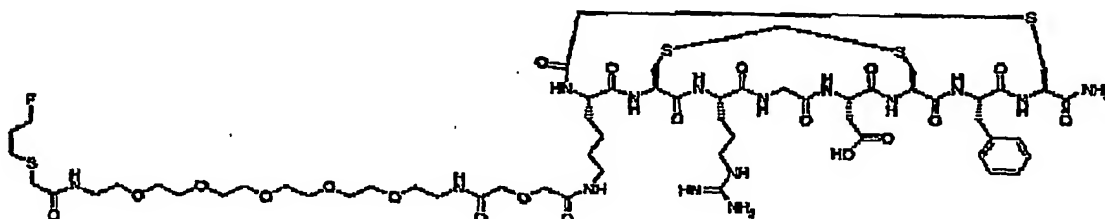
Fmoc: 9-fluorenylmethoxycarbonyl

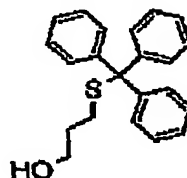
TIS: triisopropylsilane

Examples

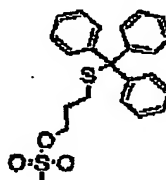
Example 1: Preparation of a 3-fluoro-propylsulfanyl labelled RGD containing peptide

Title compound:



1a) Synthesis of 3-tritylsulfanyl-propan-1-ol

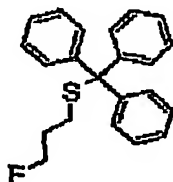
Trityl chloride (27.9 mg, 0.1 mmol) and triethyl amine (49 μ l, 0.5 mmol) were dissolved in DCM (2 ml) before 3-mercapto-1-propanol (9 μ l, 0.1 mmol) was added. DCM was evaporated under reduced pressure after 6 hours and the crude product purified by reverse phase preparative chromatography (Vydac 218TP1022 column; solvents A= water / 0.1% TFA and B= CH₃CN / 0.1% TFA; gradient 30-70 % B over 40 min; flow 10 ml / minute; detection at 254 nm). A yield of 6 mg of purified material was obtained (analytical HPLC: column phenomenex Luna C18,00B-4251-E0; solvents: A= water / 0.1% TFA and B= CH₃CN / 0.1% TFA; gradient 30-70 % B over 10 min; flow 1.0 ml / minute; retention time 7.73 minutes detected at 214 and 254 nm). Structure verified by NMR.

1b) Synthesis of methanesulfonic acid 3-tritylsulfanyl-propyl ester

15

Mesyl chloride (6 μ l, 0.075 mmol) was added to a solution of 3-tritylsulfanyl-propan-1-ol (5 mg, 0.015 mmol) and triethyl amine (32 μ l, 0.23 mmol) in THF (4 ml). After 30 minutes THF was evaporated under reduced pressure and the crude product dissolved in DCM, washed with a saturated solution of sodium hydrogencarbonate in water, a saturated solution of sodium chloride and dried with MgSO₄. A yield of 10 mg was obtained after evaporation under reduced pressure (analytical HPLC: column Luna C18,00B-4251-E0; solvents: A= water / 0.1% TFA and B= CH₃CN / 0.1% TFA; gradient 40-80 % B over 10 min; flow 1.0 ml / minute; retention time 7.12 minutes detected at 214 and 254 nm). Structure verified by NMR.

25

1c) Synthesis of (3-fluoro-propylsulfanyl)triphenylmethane

Potassium fluoride (1.4 mg, 0.024 mmol) and Kryptofix 222 (9.0 mg, 0.024 mmol) were dissolved in acetonitrile (0.2 ml) (heating). Methanesulfonic acid 3-tritylsulfanyl-propyl ester (5 mg, 0.012 mmol) in acetonitrile (0.2 ml) was added. The reaction mixture was heated to 80 degrees for 90 minutes. The crude product was purified by reverse phase preparative chromatography (Vydac 218TP1022 column; solvents A= water / 0.1% TFA and B= CH₃CN / 0.1% TFA; gradient 40-90 % B over 40 min; flow 10 ml / minute; detection at 254 nm). A yield of 2 mg of purified material was obtained (analytical HPLC: column Phenomenex Luna C18, 00B-4251-E0; solvents: A= water / 0.1% TFA and B= CH₃CN / 0.1% TFA; gradient 40-80 % B over 10 min; flow 1.0 ml / minute; retention time 8.2 minutes detected at 214 and 254 nm). Structure verified by NMR.

1d) Synthesis of Fmoc-Lys(Boc)-Cys(StBu)-Arg(Pmc)-Gly-Asp(OtBu)-Cys(StBu)-Phe-Cys(Trt)-Rink Amide AM resin.

The title peptide sequence was synthesised on a ABI 433A automatic peptide synthesiser starting with Rink Amide AM resin on a 0.1 mmol scale using 1 mmol amino acid cartridges. The amino acids were pre-activated using HBTU before coupling.

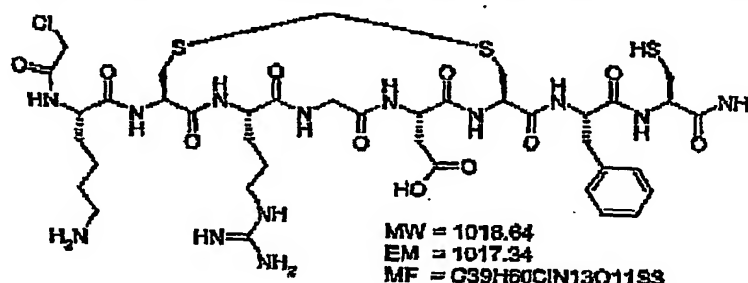
1e) Synthesis of Fmoc-Lys(Boc)-cyclo[Cys(CH₂)-Arg(Pmc)-Gly-Asp(OtBu)-Cys]-Phe-Cys(Trt)-Rink Amide AM resin.

0.05 mmol of the peptidyl resin prepared as described in 1d) was treated with a solution of 346 µL tributylphosphine, 100 µL water and 2 mL dimethylformamide. The reagents were removed after 90 minutes and the resin washed with

dimethylformamide and dichloromethane. The resin was then treated with a solution of 63 mg tetrabutylammoniumfluoride and 2 mL dichloromethane. The reagents were removed by filtration after 2 hours and the resin washed several times with dichloromethane.

5

1f) Synthesis of Cl-CH₂CO-Lys-cyclo[Cys(CH₂)-Arg-Gly-Asp-Cys]-Phe-Cys-NH₂.



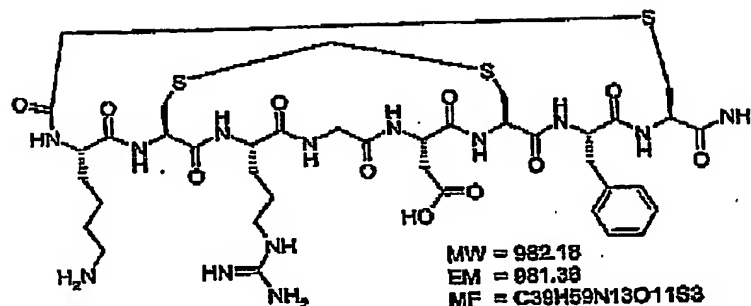
The 9-fluorenylmethoxycarbonyl group was removed from the peptidyl resin of 1e) and N-terminal chloroacetylated using chloroacetic acid anhydride. The simultaneous removal of peptide and side-chain protecting groups from the resin was then carried out in 5 mL of trifluoroacetic acid (TFA) containing 2.5 % tri-isopropylsilane and 2.5 % water for one hour and forty minutes.

After work-up 27 mg of crude peptide was obtained (Analytical HPLC: Gradient, 0-40 % B over 10 min where A = H₂O/0.1 % TFA and B = CH₃CN/0.1 % TFA; 1 mL/min; column, Phenomenex Luna 3μ C18 (2) 50 x 4.6 mm; detection, UV 214 nm; product retention time, 7.79 min). Further product characterisation was carried out using electrospray mass spectrometry. Expected, M+H at 1018.3, found, at 1018.3).

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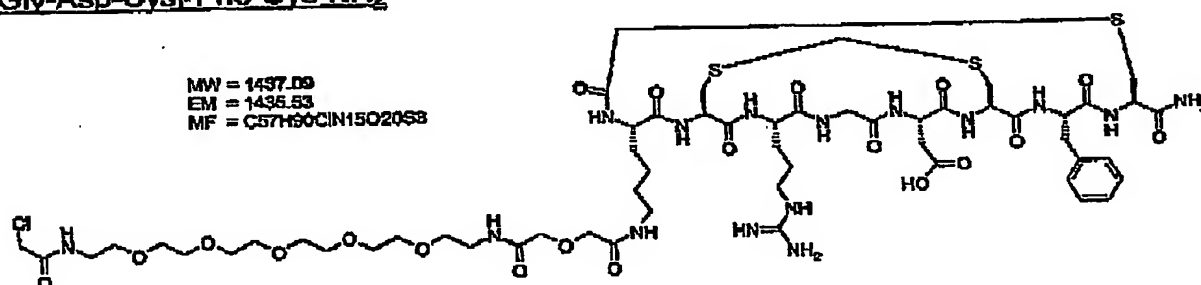
1g) Synthesis of cyclo[CH₂CO-Lys-cyclo[Cys(CH₂)-Arg-Gly-Asp-Cys]-Phe-Cys-NH₂.

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27 mg of peptide product prepared as described in 1f) was dissolved in water/acetonitrile. The mixture was adjusted to pH 8 with ammonia solution and stirred for 2 hours. After lyophilisation 26 mg of the desired product was obtained. Purification by preparative HPLC (Phenomenex Luna 5 μ C18 (2) 250 x 21.20 mm column) of the crude material was carried out using 0-30 % B, where A = H₂O/0.1 % TFA and B = CH₃CN/0.1 % TFA, over 40 min at a flow rate of 10 mL/min. After lyophilisation 9 mg of pure material was obtained. (Analytical HPLC: Gradient, 0-30 % B over 10 min where A = H₂O/0.1 % TFA and B = CH₃CN/0.1 % TFA; flow, 1 mL/min; column, Phenomenex Luna 3 μ C18 (2) 50 x 4.6 mm; detection, UV 214 nm; product retention time, 7.00 min). Further product characterisation was carried out using electrospray mass spectrometry. Expected, M+H at 982.4, found, at 982.3).

1h) Synthesis of cyclo[-CH₂CO-Lys(C1-CH₂CO-amino-PEG)-cyclo[Cys(CH₂)-Arg-Gly-Asp-Cys]-Phe-Cys-NH₂]

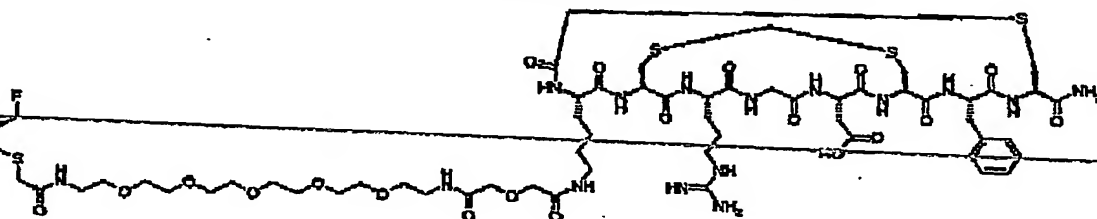


9 mg of peptide prepared as described in 1g), 34 mg Boc-amino-PEG anhydride and 7 μ L N-methylmorpholine were dissolved in 1 mL dimethylformamide and the mixture stirred for 30 minutes. The reaction was quenched by adding a solution of

25 μ L N-methylmorpholine and 2 mL water (pH \sim 9). The mixture was stirred for 2 hours, then evaporated to dryness. The residue was treated with a solution of 5 mL TFA containing 2.5 % tri-isopropylsilane and 2.5 % water for one hour. TFA was evaporated *in vacuo*, diethyl ether added to the residue and the resulting precipitate washed with diethyl ether and air-dried. The precipitate was dissolved in 3 mL dimethylformamide together with 8 mg chloroacetic acid anhydride and 9 μ L N-methylmorpholine and the mixture stirred for a further 60 minutes. The reaction mixture was evaporated to dryness.

Purification by preparative HPLC (Phenomenex Luna 5 μ C18 (2) 250 x 21.20 mm column) of the crude material was carried out using 5-50 % B, where A = H₂O/0.1 % TFA and B = CH₃CN/0.1 % TFA, over 40 min at a flow rate of 10 mL/min. After lyophilisation 5 mg of pure material was obtained. (Analytical HPLC: Gradient, 5-50 % B over 10 minutes where A = H₂O/0.1 % TFA and B = CH₃CN/0.1 % TFA; flow, 1 mL/min; column, Phenomenex Luna 3 μ C18 (2) 50 x 4.6 mm; detection, UV 214 nm; product retention time, 7.08 min). Further product characterisation was carried out using electrospray mass spectrometry. Expected, M+H at 1436.5, found, at 1436.0).

20 1i) Site-specific conjugation to the chloroacetyl modified peptide

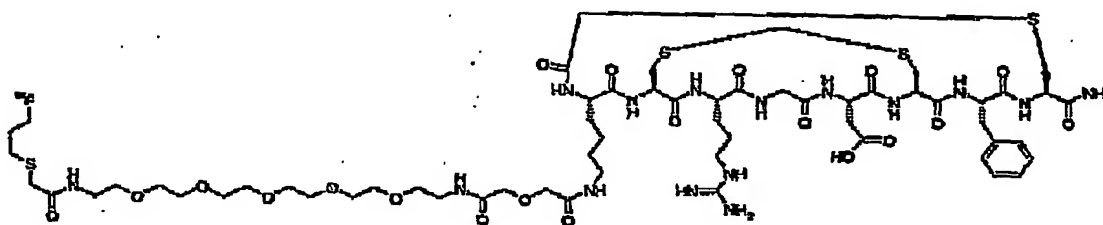


The trityl group from (3-fluoro-propylsulfanyl)triphenylmethane (2.0 mg, 0.006 mmol) described in 1c) was cleaved with TFA (100 μ L) in the presence of TIS (10 μ L) and water (10 μ L) (5 minutes). The mixture was diluted with 250 μ L water and 250 μ L acetonitrile before a solution of α [CH₂CO-Lys(ClCH₂CO-amino-PEG)-c[Cys(CH₂)-Arg-Gly-Asp-Cys]-Phe-Cys]-NH₂ (4.0 mg, 0.003 mmol) from 1h) in 500 μ L water and 500 μ L acetonitrile was added and the pH adjusted to 10 with potassium carbonate buffer (ca 400 μ L). The reaction mixture was heated to 60°C

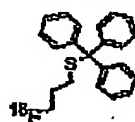
for 50 minutes. The reaction mixture was quenched with TFA and purified using reverse phase preparative chromatography (Phenomenex, C18, 00G-4253-N0 column; solvents A= water / 0.1% TFA and B= CH₃CN / 0.1% TFA; gradient 10-50 % B over 30 min; flow 5 ml / minute; detection at 254 nm). A yield of 1.1 mg of purified material was obtained (analytical HPLC: column Vydac 218TP54: solvents: A= water / 0.1% TFA and B= CH₃CN / 0.1% TFA; gradient 10-50 % B over 10 min; flow 1.0 ml / minute; retention time 13.20 minutes detected at 214 and 254 nm). Further characterisation was carried out using mass spectrometry, giving m/z value 1494.1. [M-H]⁺ as expected for the desired product.

Example 2: Preparation of a 3-[¹⁸F]-fluoro-propylsulfanyl labelled RGD containing peptide

Title compound:



2a) Preparation of ¹⁸F synthon: (3-[¹⁸F]fluoro-propylsulfanyl)triphenylmethane



To a Wheaton vial (2 ml) charged with Kryptofix[®] 222 (10 mg), potassium carbonate (1 mg dissolved in 50 µl water), and acetonitrile (0.8 ml) the fluorine-18 containing water (10 mCi, 1 ml) was added. The solvent was removed by heating at 110 °C for one hour under a stream of nitrogen. Anhydrous acetonitrile (0.5 ml) was added and again evaporated as before. This step was repeated twice. The vial was cooled to room temperature followed by injecting a solution of mesylate prepared as described in Example 2 b) (1 mg) in anhydrous DMSO (0.2 ml). The reaction mixture was stirred at 80 °C for 5 min and analysed by HPLC (gradient 1, radiochemical yield 90 %).

The reaction mixture was diluted with DMSO/water (1:1 v/v, 0.15 ml) and loaded onto a SepPak-Plus cartridge (C18, Waters) that had been conditioned (10 ml acetonitrile, 20 ml water). The cartridge was washed with water (10 ml) and the product eluted using acetonitrile. The radiochemical purity was 99 %.

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2b) Site-specific conjugation to the chloroacetyl modified peptide

Deprotection of (3-[¹⁸F]fluoro-propylsulfany)triphenylmethane, prepared as described in 2a) and subsequent reaction with the chloroacetyl modified peptide, prepared as described in 1h) is effected using methods analogous to those described in Example 1i).

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Biological Data

Using cell membrane preparations known to express the $\alpha v \beta 3$ integrin receptor, competitive binding studies were carried out using ¹²⁵I-echistatin and the F-labelled peptides as competing ligand. Binding curves were obtained and K_i's calculated using Prism™ software.

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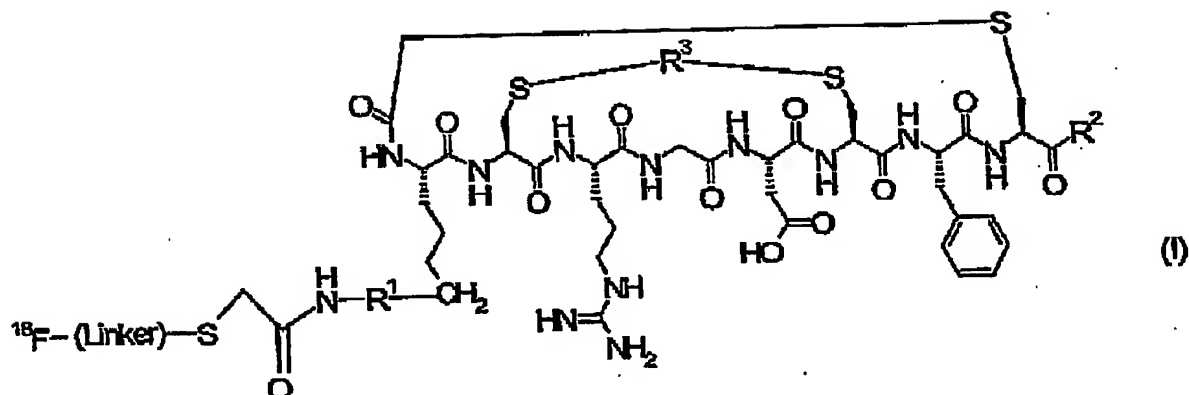
The compound of Example 1, had a K_i of 7nmole.

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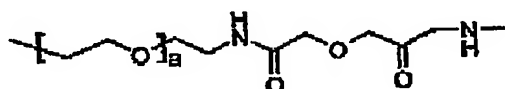
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Claims

- 5 1. A compound of formula (I):

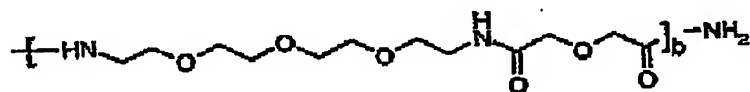


- 10 wherein
 R^1 is either a bond or is



wherein a is an integer of from 1 to 30;

- 15 R^2 is



wherein b is an integer of from 0 to 10;

- 20 R^3 is a C_{1-4} alkylene or C_{2-4} alkenylene bridge;

the Linker is a C_{1-30} hydrocarbyl group optionally including 1 to 10 heteroatoms.

2. A compound of formula (I) according to claim 1 in which:

R^3 is C_{1-4} alkylene;

a is an integer of from 1 to 10; and

b is 1.

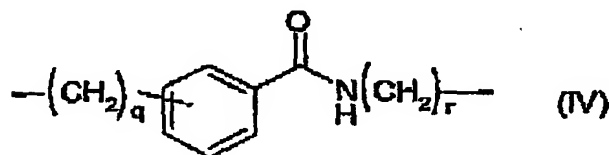
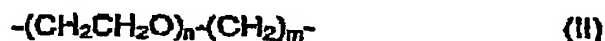
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3. A compound of formula (I) according to claim 1 or 2 in which:

R^3 is $-CH_2-$; and

a is 5.

- 10 4. A compound of formula (I) according to any of claims 1 to 3 in which the Linker is selected from (II), (III) and (IV) :



wherein:

n is an integer of 1 to 20;

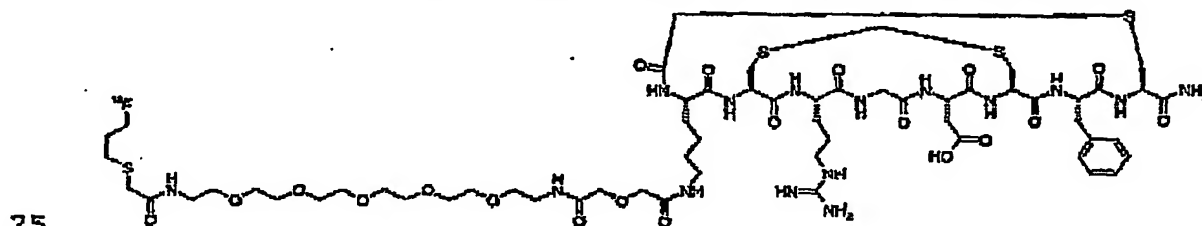
m is an integer of 1 to 10;

20 p is an integer of 1 to 20;

q is an integer of 0 to 4;

r is an integer of 1 to 10.

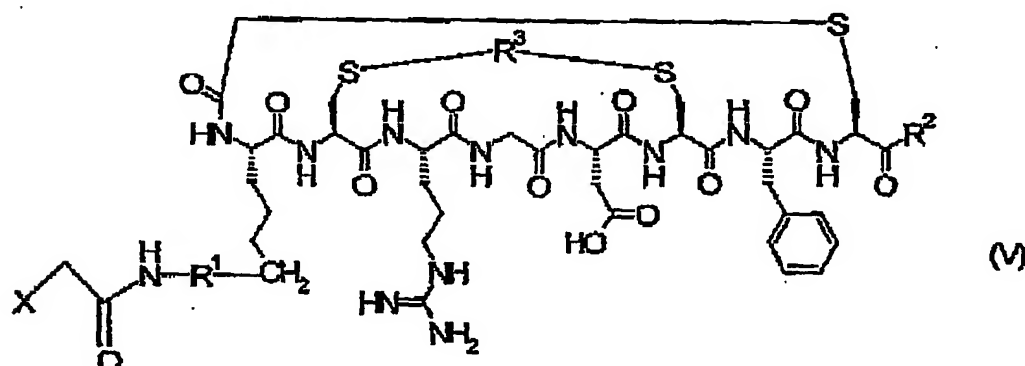
5. A compound of formula (I) according to any of claims 1 to 4 which is:



6. A compound of formula (I) according to any of claims 1 to 5 for use in

medicine, particularly in the *in vivo* diagnosis or imaging, for example by PET, of a disease or condition associated with angiogenesis.

7. A method for *in vivo* diagnosis or imaging of a disease or condition associated with angiogenesis which comprises the step of administering a compound of formula (I) according to any of claims 1 to 5 to a human or animal body, followed by generation of an image, suitably a PET image, of part or all of said body
8. A radiopharmaceutical formulation comprising a compound of formula (I) according to any of claims 1 to 5 and one or more pharmaceutically acceptable excipients.
9. A method of preparing a compound of formula (I) as defined in any of claims 1 to 5 which comprises reaction of the corresponding compound of formula (V):



- wherein R^1 , R^2 , and R^3 are as defined for the compound of formula (I) and X is a leaving group selected from chloro, bromo, and iodo, and is preferably chloro; by reaction with the appropriate compound of formula (VI):



wherein the Linker is as defined for the compound of formula (I).

10. A compound of formula (V) as defined in claim 9.

11. A kit for the preparation of a radiofluorinated peptide of formula (I) according to any of claims 1 to 5 comprising:

(i) a compound of formula (Via)



wherein L is a leaving group such as p-toluenesulphonate, trifluoromethanesulphonate, or methanesulphonate,

the Linker is a C₁₋₃₀ hydrocarbyl group optionally including 1 to 10 heteroatoms;

10 R is hydrogen or a thiol protecting group;

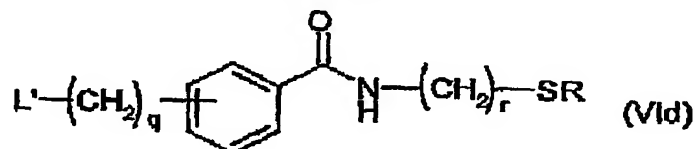
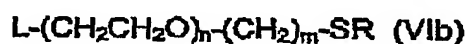
and

(ii) an activated peptide of formula (V) as defined in claim 9.

12. A kit according to claim 11, comprising:

15

(i) a compound of formula (Vib), (Vic), or (Vid):



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n is an integer of 1 to 20;

m is an integer of 1 to 10;

p is an integer of 1 to 20;

25 q is an integer of 0 to 4;

r is an integer of 1 to 10;

L is a leaving group such as p-toluenesulphonate, trifluoromethanesulphonate, or methanesulphonate;

30 L' is a leaving group such as iodo, p-toluenesulphonate, trifluoromethanesulphonate, or methanesulphonate and when q is 0, L' can be

nitro or an iodonium or ammonium salt,
R is hydrogen or a thiol protecting group; and

(ii) an activated peptide of formula (V) as defined in claim 9.

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